Manganese-Enhanced MRI of Human Choroidal Melanoma Xenografts

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PURPOSE. To test the hypothesis that the structure and function of an experimental human choroidal melanoma xenograft and neighboring non–tumor-bearing retina can be simultaneously assessed by using manganese-enhanced MRI (MEMRI).

METHODS. Spheroids grown from the human choroidal melanoma cell line C918 were implanted in the superior suprachoroidal space of 11 WAG/Nij-rnu nude rats. Two weeks later, MRI data were collected 4 hours after intraperitoneal injection of saline or MnCl2, an MRI contrast agent that can act as a biomarker of cellular demand for ions, such as calcium. The following parameters were measured: (1) tumor signal intensity, (2) inner and outer retinal signal intensity in non–tumor-bearing inferior retina, and (3) whole and inner retinal thickness of inferior retina. Separate MEMRI experiments were performed on spheroids in vitro after MnCl2 exposure and washing.

RESULTS. In vitro, spheroids exposed to MnCl2 retained sufficient Mn2+ to demonstrate contrast enhancement during MEMRI. In vivo, injection of MnCl2 resulted in a 30% increase in tumor signal intensity compared with tumors in rats injected with saline (P < 0.05). In inferior retina of tumor-bearing eyes, outer retinal signal intensity increased by 17% relative to a similar region in control eyes (P < 0.05), but there was no change in the inner retinal thickness. Total retinal thickness of the inferior retina in the tumor-bearing eyes increased by 8%, compared with that in the non–tumor-bearing eyes (P < 0.05).

CONCLUSIONS. The present identification of regions of enhanced Mn2+ uptake in choroidal melanoma and a somewhat unexpected edema and increased outer retinal ion demand in neighboring non–tumor-bearing retina highlights MEMRI as a potentially powerful method for noninvasively monitoring tumor progression and treatment response and efficacy. (Invest Ophthalmol Vis Sci. 2007;48:963–967) DOI:10.1167/iovs.06-1156

Materials and Methods

Animals

The mutant rat strain WAG/RijHsl-rnu was used in this study, because these rats are athymic and permit the xenotransplantation of human tumor tissue into the choroid.15 The WAG/RijHsl-rnu rats were bred and housed in the Department of Laboratory Animal Resources (DLAR) facility at Wayne State University. All procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Wayne State University Animal Investigation Committee.

Human Choroidal Melanoma Cell Line

The human choroidal melanoma cell line C918 was used in this study. This cell line was derived from a patient’s tumor at the University of Iowa in the 1990s.13 The cells were maintained in RPMI medium plus 10% fetal bovine serum (FBS) + antibiotic. We have demonstrated the reliable ability of these cells to generate tumor xenografts in the choroid of WAG/RijHsl-rnu nude, athymic rats.15
Growth of Tumor Spheroids
C918 tumor spheroids (three-dimensional aggregations of tumor cells) were grown by using a modified version of the method described by Yuhas et al. Agar (1%) was prepared in RPMI medium by sterilization for 30 minutes. While still hot, 20 mL of 1% agar was poured into the bottom of sterile 100 × 20 mm plastic Petri dishes. To initiate spheroid growth, 3 × 10^6 cells in 20 mL of RPMI + FBS + antibiotic were placed in each agar-coated Petri dish. The dishes were then placed in the incubator. Spheroids were implanted into the choroid when they were 200 to 500 μm in diameter and were used in the Mn^{2+} uptake study when they had been growing for 10 or 13 days.

Growth of Orthotopic Human Choroidal Melanoma Xenografts
C918 spheroids were implanted into the suprachoroidal space of rats as detailed previously. Tumor implantation was performed under sterile conditions in a BSL2 safety hood in the Wayne State DLAR facility. Rats were anesthetized with a ketamine-xylazine mixture (70/8 mg/kg IP) and proparacaine HCl (0.5%) was applied topically to the right eye as local anesthesia. The rat was placed under an operating microscope, and approximately five tumor spheroids in ≤1 μL of medium were injected into the suprachoroidal space with a 5-μL glass syringe (Model 659RR; Hamilton Co., Reno, NV) equipped with a three-quarter-inch-long beveled 32-gauge needle (cat. no. 0160832, point style 4; Hamilton Co.). Antibiotic ointment was applied to the eye, and the rat was allowed to recover on the heating blanket before being returned to its cage. C918 spheroids were implanted in the right eyes of 11 rats.

MEMRI of Rat Eyes
After 14 days of tumor growth, awake rats were injected intraperitoneally on the right side with either 44 mg/kg MnCl₂ (0.1 M solution; n = 6) or an equivalent volume of saline (n = 5) and kept in the light. After another 3.5 hours, rats were anesthetized with urethane (36% solution, intraperitoneally, 0.085 mL/20 g animal weight, prepared fresh daily; Aldrich, Milwaukee, WI). To maintain the core temperature, a recirculating heated water blanket was used. Rectal temperatures were continuously monitored throughout each experiment, as previously described. MRI data were acquired on a 4.7-T system (Avance; Bruker, Karlsruhe, Germany) using a two-turn transmit-receive surface coil (1.0-cm diameter) placed over the eye. In all rats, left and right eyes were studied sequentially. Images were acquired using an adiabatic spin-echo imaging sequence (repetition time [TR] 350 seconds, echo time [TE] 16.7 ms, number of acquisitions [NA] 16, matrix size 256 × 512, slice thickness 600 μm, field of view 12 × 12 mm², 23 minutes/image). A single transverse slice through the center of each eye (based on sagittal localizer images collected using the same adiabatic pulse sequence) was obtained for each rat.

Determination of Tumor Signal Intensity
Average signal intensities within the tumor were measured from the high-resolution MRI data of the tumor-bearing right eyes for all 11 rats, whether they received MnCl₂ or saline. The tumor area was selected and measured, and signal intensity was analyzed (Image; a freeware program available by ftp at zippy.nimh.nih.gov; or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD) and derived macros. We controlled for changes in receiver gain differences between animals by setting the signal intensity of a fixed region of noise in each rat to a fixed value. Average tumor signal intensities were chosen based on earlier in vitro studies investigating Mn²⁺ uptake by lymphocytes and pancreatic islets. The spheroids were resuspended every 10 minutes by agitation of the tubes. After the 1-hour exposure, the spheroids were washed, resuspended in RPMI + 10% FBS, and put on ice. The microcentrifuge tubes were placed in the magnet, and MRI data were obtained. This experiment was performed in triplicate.

Statistical Analysis
Since the groups were relatively small and a normal distribution could not always be assumed, average tumor and retinal signal intensities were compared using the Mann-Whitney test. Similarly, the average retinal thickness and retinal intensity data of the control and tumor-bearing eyes were compared using the nonparametric Wilcoxon signed rank test. In all tests, P < 0.05 was considered statistically significant.

RESULTS

MEMRI of C918 Tumor Spheroids
After C918 spheroids had been grown for 10 or 13 days, they were washed, resuspended in RPMI medium, and divided into microcentrifuge tubes. Spheroids were then exposed to 0, 25, or 100 μM MnCl₂ in RPMI medium + 10% FBS for 1 hour at 37°C. These MnCl₂ concentrations were chosen based on earlier in vitro studies investigating Mn²⁺ uptake by lymphocytes and pancreatic islets. The spheroids were resuspended every 10 minutes by agitation of the tubes. After the 1-hour exposure, the spheroids were washed, resuspended in RPMI + 10% FBS, and put on ice. The microcentrifuge tubes were placed in the magnet, and MRI data were obtained. This experiment was performed in triplicate.

Determination of Intraretinal Signal Intensity
Intraretinal signal intensities were measured from the high-resolution images of both eyes in the manganese-injected rats and the tumor-bearing eyes of the saline-injected rats. For visualization and analysis purposes, in-house-written software was used to map the in situ image into a linear representation of each retina. Within each group, linearized retinas were either analyzed separately or averaged into a composite image. The inner and outer inferior retinal signal intensity data from 0.4 to 1 mm from the optic nerve were extracted from the inferior control or non–tumor-bearing retina for further analysis.

Determination of Retinal Thickness
From the MEMRI data, inner or total retinal thicknesses were measured as the radial distance between the anterior and middle edge (defined by its change in signal intensity) or posterior edges of the retina. Six measurements at distances between 0.4 and 1 mm from the optic nerve were measured in the inferior retina of each image. Inner retinal thickness could not be determined in one set of eyes, because the rat may have been dark-adapted and the inner–outer retina border was not distinguishable. Mean inferior values generated for each rat were used for paired comparisons.

For the saline-injected rats (i.e., without MnCl₂ injection), total retinal thicknesses in the inferior retina were measured as described earlier. Inner retinal thicknesses could not be measured, since the inner–outer retina is not readily distinguishable without Mn²⁺.

MEMRI of C918 Human Choroidal Melanoma Spheroids
After exposure to 100 μM MnCl₂, spheroid signal intensity increased by 30% ± 22% (mean ± SD, n = 3), which is indicative of intracellular retention of Mn²⁺ (Fig. 1).

FIGURE 1. Representative high-resolution MRIs (46.8 × 23.4 × 600 μm³) of C918 spheroids 4 hours after exposure to MnCl₂ for 1 hour at 37°C. The spheroids were pooled at the bottom of the tube.
High-Resolution MRI of C918 Human Choroidal Melanoma Xenografts without MnCl₂ Injection

High-resolution, T₁-weighted images of the right eye revealed the presence of a C918 xenograft in the superior portion of the eye (Fig. 2A). Tumors were identified in all five rats injected with saline and appeared hyperintense relative to the retina and vitreous. In-plane tumor areas ranged from 1.5 to 3.2 mm², with an average area of 2.25 ± 0.76 mm² (mean ± SD, n = 5; Table 1).

High-Resolution MEMRI of C918 Human Choroidal Melanoma Xenografts with MnCl₂ Injection

Signal enhancements consistent with retention of Mn²⁺ were evident within all six tumors in rats injected with MnCl₂ (Fig. 2B). In-plane tumor area ranged from 1.5 to 6.4 mm², with an average area of 3.89 ± 2.33 mm² (mean ± SD, n = 6; Table 1). There was no difference between the areas of the tumors injected with saline and those injected with MnCl₂ (Table 1, P = 0.537). In the MnCl₂-injected rats, the average intensity of the tumors was 30% greater than the corresponding value in the saline-injected rats (Table 1, P = 0.030).

MEMRI of Retina in Tumor-Bearing Rats

In the rats injected with MnCl₂, signal enhancements were evident throughout the retina in both the tumor-bearing right eye (Fig. 2) and the control left eye. In the tumor-bearing right eye, the signal intensities of the inner layer of the inferior retina (178.5 ± 7.5 AU, n = 6) and outer retina (182.7 ± 9.3 AU, n = 6) were significantly greater after injection of MnCl₂ than the corresponding values in the inferior retina after injection of saline (inner retina: 76.4 ± 11.0 AU, outer retina: 74.8 ± 7.2 AU, n = 5, P = 0.004).

As expected for light-adapted control retinas after injection of MnCl₂, the average signal intensity of the inner layer of the inferior retina of the control eyes was significantly greater than the intensity of the outer retina (Fig. 3A, Table 2). In contrast, in the tumor-bearing eyes, inner and outer retina signal intensities were similar (Fig. 3B, Table 2).

Visual comparison of the linearized spatial maps of average signal intensity of the inferior retina in each group revealed differences in the Mn²⁺ enhancement patterns (Fig. 3). The intensity of the non–tumor-bearing inferior outer retina in the tumor-bearing eyes was 16.5 ± 7.6% (mean ± SD, n = 6, P = 0.031) higher than that of the outer retina in the corresponding control eyes (Fig. 3, Table 2). In contrast, no difference was found in the average intensities of the non–tumor-bearing inferior inner retinas of the tumor-bearing eyes compared with the control eyes (Table 2; P = 0.563). Similarly, in the absence of Mn²⁺, the signal intensity of the inferior inner retina (76.4 ± 11.0 AU, n = 5) was not significantly different from that of the outer retina (74.8 ± 7.2 AU, n = 5, P = 0.813) in the tumor-bearing eyes.

Retinal Thickness

The total retinal thickness of the inferior retina in the tumor-bearing eye was 7.7% ± 9.5% greater than that of the corresponding control eye (Table 2, P = 0.031). There was no difference between the thickness of the inferior inner retina of the tumor-bearing eye and that of the corresponding control eye (Table 2). The total thickness of the inferior retina in the tumor-bearing eye of the saline-injected rats (253.3 ± 4.8 μm; n = 5), was not significantly different from the thickness of the inferior retina in the MnCl₂-injected rats (246.1 ± 21.8 μm; n = 6, P = 0.082).

DISCUSSION

This study has resulted in two significant new findings. First, tumor cells were shown to retain a significant amount of Mn²⁺ after exposure in vitro and in vivo, resulting in MRI signal enhancement. Second, it was demonstrated that the structure and function of neighboring non–tumor-bearing retina was affected by the presence of the melanoma. These data lay the foundation for future studies of treatment efficacy for choroidal melanoma.

High-Resolution MEMRI of C918 Human Choroidal Melanoma Xenografts

As expected, high-resolution MRI imaging of the rat eye, even without infusion of MnCl₂, permitted the visualization of hu-

![Figure 2](image-url)  ![Figure 3](image-url)

**FIGURE 2.** Representative high-resolution MRIs of tumor-bearing eyes 4 hours after IP saline injection (A) or MnCl₂ injection (B). Comparison of peripheral superior and inferior retina clearly identifies the tumors (large arrows). The tumor areas were (A) 1.54 and (B) 1.87 mm². The average intensities of these tumors were (A) 122.1 AU and (B) 167.5 AU. Note that the data in Figure 3 was derived from the region of inferior retina located between the small arrows.

**FIGURE 3.** Pseudocolor images of average retinal signal intensity in the inferior retina (see Fig. 2) of the control left eye (A) and the inferior retina of the tumor-bearing right eye (B). The same pseudocolor scale was used for both images, where blue to green to yellow to red represent lowest to highest signal intensity. Top dotted line: presumed boundary between the inner and outer retina of the control eye; bottom dotted line: boundary of the posterior aspect of the control eye.

<table>
<thead>
<tr>
<th>Injection</th>
<th>In-Plane Tumor Area (mm²)</th>
<th>Average Signal Intensity (AU)</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.25 ± 0.76</td>
<td>114.1 ± 13.1</td>
<td>5</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>3.89 ± 2.33</td>
<td>148.9 ± 25.5</td>
<td>6</td>
</tr>
<tr>
<td><em>P</em></td>
<td>0.537</td>
<td>0.030</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Average Signal Intensity of Tumor after Saline or MnCl₂ Injection

Data are expressed as the mean ± SD.

* Saline- versus MnCl₂-injected rats; Mann-Whitney rank sum test.

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man choroidal melanoma xenografts growing in the suprachoroidal space in this nude rat model. As is the case with most choroidal melanomas in humans,12 the orthotopic xenografts were hyperintense compared with the intensity of the vitreous (Fig. 2). Injection of MnCl₂ greatly enhanced the tumor-retina contrast.

The present finding of substantial intraocular tumor uptake of Mn²⁺ is consistent with the results of a previous nonocular study, in which the uptake of radioactively labeled Mn²⁺ was five times higher in a brain tumor than in the normal surrounding brain.13 However, that study did not determine whether the free Mn²⁺ ion was within the tumor cells or was extracellular. To investigate, we examined Mn²⁺ uptake by tumor spheroids in vitro. The C918 cells clearly internalized the Mn²⁺, since, even after thorough rinsing, exposure of the spheroids to MnCl₂ resulted in contrast enhancement (Fig. 1). Although Mn²⁺ accumulation has been related to increased neuronal activity9–11 and increased pancreatic islet function,20 the exact mechanism behind the increased uptake in tumors is not known. Because Mn²⁺ can enter cells through voltage-gated and voltage-independent calcium channels,22,23 it may serve as a surrogate marker of Ca²⁺ uptake. Since Ca²⁺ uptake is associated with increased proliferation,22,25 the increased Mn²⁺ accumulation may mimic increased Ca²⁺ uptake by mitotically active tumor cells. Alternatively, because Ca²⁺ also plays a role in angiogenesis,26 the increased Mn²⁺ uptake could be a marker of sites of tumor-induced angiogenic activity. Future studies are needed to elucidate the mechanism of Mn²⁺ accumulation in the tumor cells.

Effects of the Presence of the Tumor on Neighboring Retina

One of the major advantages of using MEMRI in this xenograft model is the ability to obtain simultaneous structural and functional information on the tumor and on the neighboring retina in the inferior non–tumor-bearing portion of the eye. In the control left eye, the inferior inner retinal intensity was greater than that in the inferior outer retina (Fig. 3; Table 2), which agrees with earlier results in the normal light-adapted rat retina.²³ In contrast, in the tumor-bearing eye, we found high Mn²⁺ uptake in both the inferior inner and outer layers of the retina, which is the pattern typically found in dark-adapted conditions,²⁴ even though these animals were light adapted. In particular, signal intensity or Mn²⁺ accumulation increased in the inferior outer retina of the tumor-bearing eyes compared with the control eyes (Table 2). The mechanism behind this increase in outer retinal Mn²⁺ uptake is not known. It may be caused by a tumor-induced change in intraocular pressure that could alter retinal function. Alternatively, it could be attributable to the direct effect of molecules released by the tumor on the retinal tissue. Regardless of the mechanism behind the Mn²⁺ accumulation, the function of the inferior outer retina, as assessed by ion demand, was adversely affected by the presence of the tumor, even though this section of retina was not directly involved in the tumor growth.

It was also demonstrated that the presence of the tumor in the superior retina caused an increase in the total thickness of the inferior retina, suggestive of edema (Table 2). The mechanism behind this edema is unclear, but it may be related to a secondary effect of the tumor on ocular function or the direct effect of secretory products on the retina.

The ability to monitor retinal function and thickness simultaneously in the tumor-bearing eye is an important advantage of MEMRI imaging in this model. We anticipate that MEMRI will allow the determination of bystander effects of tumor growth on the neighboring retina. Perhaps more important, with this technique, the impact of various treatments on the tumor and on the neighboring retina can be evaluated. Minimizing collateral damage to the retina is a serious issue in current treatments and in the development of new treatment modalities for choroidal melanoma.⁴–⁶

### Summary

For the first time, systemic injection of a MnCl₂ solution has been used to image a tumor by using MEMRI. The imaging of a human choroidal melanoma xenograft transplanted into the eye of a nude rat permitted the simultaneous evaluation of the tumor and the neighboring retina in the same eye. Mn²⁺ was taken up by the tumor, which resulted in contrast enhancement during MEMRI imaging. The retina in the inferior portion of the eye, which was not in direct contact with the tumor, was adversely affected by the presence of the melanoma. Inferior outer retinal MEMRI intensity was altered in those eyes, and the inferior retina was edematous. The use of the MEMRI technique in this choroidal melanoma model will permit the study of the impact of tumor growth on the neighboring retina and will allow the simultaneous evaluation of treatment-related side effects in the same eye.

The practical applicability of this method in humans has not yet been evaluated. However, we note that a manganese-based contrast agent (Mangafodipir Trisodium [Teslascan]; GE Healthcare, Princeton, NJ) already has FDA approval and that motion–artifact free high-resolution MRIs of the human retina can be routinely collected.²⁷–³⁰ These considerations raise the possibility of clinically monitoring choroidal melanoma progression and/or treatment response using MEMRI in patients.

### Acknowledgments

The authors thank Mary Hendrix and Karla Daniels for kindly supplying the C918 cells.

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**Table 2. Average Retinal Thickness and Average Signal Intensity of the Inferior Retina in the Control Left Eye and the Tumor-Bearing Right Eye after MnCl₂ Injection**

<table>
<thead>
<tr>
<th>Eye</th>
<th>Total Inferior Retinal Thickness (µm)</th>
<th>Inner Inferior Retinal Thickness (µm)</th>
<th>Outer Retinal Intensity (AU)</th>
<th>P Inner vs. Outer Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>228.6 ± 6.9</td>
<td>107.7 ± 3.2</td>
<td>181.0 ± 11.5</td>
<td>0.031</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>246.1 ± 21.8</td>
<td>118.6 ± 15.4</td>
<td>178.5 ± 7.3</td>
<td>0.438</td>
</tr>
<tr>
<td>Rats (n)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.031</td>
<td>0.188</td>
<td>0.563</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Control versus tumor-bearing eyes or inner and outer retina; Wilcoxon signed rank test.

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Reference numbers such as 23, 24, and 25 should be updated to reflect the current citation style if necessary.
References